without prejudice to their presentation in an appropriately-filed divisional application.

The amendments to claim 1 are supported by originally-filed claim 1, and at page 17, lines 16-23 of the specification.

Amended claims 3-4, 6-9 and 11 are supported by originally-filed claims 3-4, 6-9 and 11, respectively.

The objections to claims 3-12 at items 5 and 6 of the Office Action are moot in view of the amendments to the claims.

The 35 U.S.C. § 101 Rejection

The Examiner rejected claims 1 and 3-10 under 35 U.S.C. § 101 as directed to non-statutory subject matter. The amendment to claim 1, to recite "isolated and purified", renders the rejection moot. Hence, the Examiner is requested to withdraw the § 101 rejection of the claims.

The 35 U.S.C. § 112, Second Paragraph, Rejection

The Examiner rejected claims 1 and 3-12 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The amendments to the claims overcome this rejection, and so withdrawal of the § 112(2) rejection of the claims is respectfully requested.

The 35 U.S.C. § 112, First Paragraph, Rejection

The Examiner rejected claims 1 and 3-12 under 35 U.S.C. § 112, first paragraph, as the specification allegedly fails to enable chemokine peptides other than those set forth in SEQ ID numbers 1 and 7-14 and the CRD peptide set forth in SEQ ID NO:14. This rejection is respectfully traversed.

The claims are directed to an isolated and purified peptide of a chemokine, a variant or a derivative thereof, which peptide comprises at least three contiguous residues, and no more than 30 residues, corresponding to residues in the carboxy-terminal half of the mature form of the chemokine, and which peptide inhibits the activity of the corresponding native chemokine. The specification refers to the claimed peptides as chemokine peptide 3, a variant thereof, or a derivative thereof.

As evidence that Applicant's disclosure would enable the art worker to prepare chemokine peptides, variants or derivatives thereof, falling within the scope of the claims, the Examiner is respectfully requested to consider Applicant's detailed specification. It is disclosed that a chemokine peptide 3 of the invention may comprise as few as three contiguous residues, and no more than 30 amino acid residues, which correspond to chemokine sequences generally located in the carboxyl-terminal half of the chemokine (see page 5, lines 11-22 and Example 6). For example, Table 3 shows an alignment of selected chemokines and indicates the general location of peptide 3 in human MCP-1, murine MCP-1, human MCP-2, human MCP-3, human MIP-1α, human MCP-1β, RANTES, eotaxin, IL8 and human SDF-1α. It is disclosed that cysteine residues are used to generally align the sequences. Exemplary chemokines, from which the peptides of the invention may be obtained or derived, are listed at page 17, lines 3-15 of the specification, and include MCP-1, MCP-2, MCP-3, MIG, MIP1α, MIP1β, RANTES, PF-4, I-309, HCC-1, eotaxin, C10, CCR-2, ENA-78, GROα, GROβ, GROγ, IL-8, IP-10, SDF1α, SDF1β, TARC, LARC, MIG, Ckβ8, CCF18/MRP-2, MIPIτ, and NAP-2,

It is further disclosed that a peptide of the invention may have 100% contiguous amino acid sequence homology or identity to the amino acid sequence of a native chemokine, or have less than 100% homology to the corresponding amino acid sequence of a nature chemokine, i.e., the peptide is a "variant" peptide. A variant peptide is disclosed as a peptide which has amino acid residues not present in the corresponding wild-type chemokine, e.g., amino acid substitution(s), internal deletion(s) or D-amino acid(s). Chemokine peptides or peptide variants which are subjected to chemical modifications, such as esterification, amidation, reduction, protection and the like, are referred to as chemokine "derivatives." For example, a modification known to improve the stability and bioavailability of peptides *in vivo* is the cyclization of the peptide. Thus, a derivative of a peptide of the invention may include a cyclic reverse sequence derivative (CRD), linear reverse D derivative (LRD) and cyclized forward L derivative (CFL) of a peptide of the invention.

Moreover, the specification provides exemplary *in vitro* and *in vivo* assays to identify whether a chemokine peptide, a variant thereof, or a derivative thereof, inhibits or reduces a chemokine-induced activity (page 27, lines 25-27). These assays include *in vitro* assays (see page 28, line 9-page 30, line 10) which detect whether an agent inhibits the chemokine-induced chemotaxis of a variety of cell types (e.g., neutrophils, monocytes, eosinophils, mast cells,

platelets or lymphocytes; page 29, lines 12-13), inhibits the release of enzymes from certain cells (such as N-acetyl-β-D-glucosamidase from monocytes or elastase from neutrophils; page 30, lines 13-24), changes the concentration of cytosolic free Ca^{2+} in various cell types (monocytes, eosinophils, neutrophils; page 30, line 2-page 31, line 29), inhibits binding to a chemokine receptor and/or displaces bound chemokine (page 32, line 1-page 33, line 9), and inhibits the co-mitogenic activity of a chemokine (page 35, line 25-page 34, line 2).

Example 1 discloses the use of an *in vitro* chemotaxis assay, i.e., the inhibition of chemokine-induced THP-1 (a monocytic cell line) migration, to identify regions of human MCP-1 (hMCP1) falling within the scope of the invention. Example 4 describes that a CRD peptide variant of MCP-1 inhibited MCP-1-induced THP-1 migration. Table 4 shows the inhibition by a MCP-1 chemokine peptide 3 of the MCP-1-, MIP1α-, IL8- and SDF-1α-induced migration of THP-1 cells and primary human monocytes. Table 6 shows ED_{50} data for four chemokines (MCP-1, MIP1α, IL8 and SDF-1α) and selected peptides which include variants of MCP-1 chemokine peptide 3, e.g., one variant peptide of human MCP-1 chemokine peptide 3 (the variant is designated $Leu_4Ser_7Ile_{11}$peptide3(1-12)[MCP-1]) has amino acid substitutions at positions 4, 7 and 11 relative to the sequence of a 12 amino acid peptide of human MCP-1 designated peptide 3(1-12)[MCP-1], and another variant (referred to as $Ser_7Glu_8Glu_9$peptide3(1-12)[MCP-1]) has substitutions at positions 7, 8 and 9 relative to peptide 3(1-12)[MCP-1]. Thus, the Examiner is respectfully requested to note that, contrary to the Examiner's assertion at page 9 of the Office Action, Table 6 provides evidence that variant peptides can inhibit chemokine-induced activity.

Table 6 also includes data from three chemokine peptides having three amino acid residues, one of which is a tripeptide from MIP-1α. Some of the peptides described in Table 6 were found to be pan-chemokine inhibitors, while others showed selectivity for certain groups of chemokines, i.e., selectivity for CC or CXC chemokines. Example 6 discloses additional experiments for tripeptides of the invention. Thus, the tripeptide WVQ, a sequence found in the carboxy-terminal half of MCP-1, MCP-3, MIP-1α, MIP-1β, RANTES, eotaxin and IL8, inhibited all four chemokines tested, while tripeptide KQK, another sequence found in the carboxy-terminal half of MCP-1, was specific for MCP-1 (versus MIP-1α, IL8 or SDF-1α). It is disclosed that the corresponding tripeptides for MIP-1α (SEE), SDF-1 (KLK), and IL8 (KEN) were each specific for the cognate chemokine.

It is further disclosed that the efficacy of a peptide of the invention in an animal model may be assessed by clinical parameters specific for the particular model or by general parameters such as the extent of inflammation or cellular infiltration into affected tissues (page 44, lines 14-15). Animal models which may be employed to determine whether a peptide of the invention inhibits chemokine-induced activity *in vivo* are exemplified at pages 43-46 of the specification. For example, atherosclerosis is associated with chemokine-induced, e.g., MCP-1-induced, macrophage recruitment. Animal models of atherosclerosis include apoE knockout mice, mice which over express human apoB, and Watanabe heritable hyperlipidemic rabbits (page 44, lines 1-5). Animal models for autoimmune disease include collagen-induced arthritis in DBA/1 mice and myelin basic protein-induced experimental autoimmune encephalomyelitis. Animals models for osteoporosis include ovariectomized female rats, mice and monkeys, rats treated with heparin or glucocorticoids, and suspension-induced osteoporosis in rats. Thus, for atherosclerosis, the extent of lipid lesion formation in vessel walls may be determined in animals that have been administered a peptide of the invention relative to control animals (page 44, lines 23-27). For osteoporosis, bone density may be determined (page 78), as well as the presence bone matrix degradation products in plasma and urine (page 78), in animals that have been administered a peptide of the invention relative to control animals.

Thus, Applicant has provided a disclosure sufficient to enable the art worker to practice the invention as broadly as it is claimed.

The Examiner alleges that Applicant's specification must provide sufficient guidance to permit the art worker to identify embodiments "which are more likely to work than not, without actually making and testing them". It is well-settled that it is not necessary that a patent applicant have prepared and tested all the embodiments of his invention in order to meet the requirements of § 112. In re Angstadt, 537 F.2d 498, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976). If Applicant's invention is disclosed so that one of ordinary skill in the art can practice the claimed invention, even if the practice of the invention by the art worker includes routine screening or some experimentation, Applicant has complied with the requirements of 35 U.S.C. § 112, first paragraph. In re Angstadt, 537 F.2d 498, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976).

With respect to the "undue experimentation" alleged by the Examiner to be necessary to determine which peptides have the desired activity, the fact that the outcome of such a synthesis/screening program is unpredictable is precisely why a screening program is carried out.

The Examiner simply cannot reasonably contend that a screening program to locate biomolecules with target biological or physical properties would not be carried out by the art because the results cannot be predicted in advance.

In fact, the Federal Circuit has explicitly recognized that the need, and methodologies required, to carry out extensive synthesis and screening programs to locate bioactive molecules do not constitute undue experimentation. In re Wands, 8 U.S.P.Q.2d 1400, 1406-1407 (Fed. Cir. 1988), the Court stated:

> The nature of monoclinal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody.

Likewise, practitioners in the art related to the present application would be well-equipped to prepare and screen peptides of chemokines, amino acid substituted peptides of chemokines and derivatives thereof to locate additional peptides falling within the scope of the claims. See also, Hybritech Inc. v. Monoclinal Antibodies Inc., 231 U.S.P.Q. 81, 84 (Fed. Cir. 1986) (evidence that screening methods used to identify characteristics [of monoclinal antibodies] were available to art convincing of enablement). Thus, the fact that a given claim may encompass a large number of peptides is not dispositive of the enablement issue, particularly in an art area in which the level of skill is very high and in which screening of large numbers of compounds has been standard practice for at least ten years (Ex parte Forman, 230 U.S.P.Q.2d 456 (Bd. App. 1986).

The Examiner alleges that as the embodiments corresponding to the claimed peptides is enumerable, there are substantial scientific reasons to doubt the scope of enablement. However, the Patent Office must provide evidence inconsistent with the contested disclosure as it relates to the operability in the specification. In re Marzocchi et al., 439 F.2d 220, 169 U.S.P.Q. 367, 370 (C.C.P.A. 1971). By generally asserting that "there are substantial scientific reasons to doubt the scope of enablement", it is Applicant's position that the Examiner has failed to meet this burden, that is, the Examiner has not provided evidence inconsistent with Applicant's disclosure.

Moreover, there is no requirement that all of the compounds within a claimed genus exhibit the same degree of efficacy in order to meet the requirements of 35 U.S.C. § 112. In re Gardner, 475 F.2d 1389, 177 U.S.P.Q. 396, 398 (C.C.P.A. 1973). If Applicant has provided the art worker with sufficient guidance on how to identify those peptides, Applicant has complied

with the requirements of § 112(1). Further, the Examiner is reminded that peptides that do not inhibit the activity of a native chemokine are not within the scope of the claims.

The Examiner cites Genentech Inc. v. NovoNordisk (42 U.S.P.Q.2d 1003 (C.A.F.C. 1997)) to support the enablement rejection. The claims at issue in Genentech were directed to a method of producing a cleavable human growth hormone (hGH) fusion protein. The specification described general applications for cleavable fusion expression, an enzyme (trypsin) that might be used for cleavage, and sites recognized by that enzyme. For its analysis, the court noted that in applicant's specification, "reasonable detail must be provided in order to enable members of the public to understand and carry out the invention" (page 1005). The court found that the specification did not describe in any detail how to make hGH using a cleavable fusion protein expression system, reaction conditions under which cleavable fusion expression would work, or a specific cleavable fusion protein.

The Genentech court concluded that the claimed invention was the application of an unpredictable technology in the early stages of development, and so would required undue experimentation by the art worker to practice. Evidence to support this conclusion was that, although prior to applicant's filing the DNA sequence of desirable human proteins was known and many skilled scientists were attempting to express recombinant human protein, the successful preparation of a human protein by expressing a cleavable fusion protein did not occur until one year after applicant's filing, and it was not until five years later that the successful preparation of hGH by expressing a cleavable fusion protein was reported.

Unlike Genentech, Applicant's specification provides more than adequate detail so that the art worker understands and can carry out the invention, i.e., the identification and preparation of peptides of a chemokine, variants thereof, and derivatives thereof, that inhibit the activity of at least one chemokine. Also in contrast to Genentech, exemplary peptides within the scope of the claims are described in the specification and have biological activity. And given the data provided in the present specification, the art worker has a reasonable expectation that other chemokine peptides are likely to inhibit the activity of at least one chemokine.

To support the proposition that the substitution of an amino acid residue in a polypeptide does not lead to a reasonable expectation that the resulting substituted polypeptide will have a desired activity, the Examiner cites to Cunningham et al. (Science, 244, 1081 (1988)) and pages 145-146 of George et al. (In: Macromolecular Sequencing and Synthesis, pp. 127-149, Alan Liss,

Inc. NY (1989)). Cunningham et al. disclose that 62 single alanine substitutions were introduced into hGH at positions 2-19, 54-74 and 167-191, positions which had been implicated in receptor recognition. The authors note that "[a]lanine-scanning mutagenesis generates a small and systematic set of mutant proteins that can be readily assayed by quantitative binding analysis" (page 1081). A number of the substitutions resulted in a protein having a lower binding affinity to hGH receptor (Figure 1).

However, the fact that single amino acid substitutions of alanine in hGH may result in changes in receptor binding affinity, does not provide evidence that the substitution of amino acids in a polypeptide results in a substituted polypeptide having a property that is wholly unpredictable. Indeed, the alanine substituted hGH mutants still bound to the hGH receptor, many with a reduced affinity, some with an increased affinity, and still others with an affinity that was similar to wild-type hGH. Further, based on the binding of the mutants to eight different monoclonal antibodies and circular dichroic spectra, the authors concluded that "it is unlikely" that the substitutions caused major tertiary structural changes, but more likely "small and local structural perturbations" (page 244). Moreover, Cunningham et al. clearly illustrates that, even in 1988, it was within the skill of the art to determine whether a large number of structurally related polypeptides had a certain activity.

The George et al. paper relates to current methods for sequence comparison and analysis. While page 145 of George et al. discloses that sequence comparison methods will not be able to assess biological relatedness until the structure/function problem is more clearly understood, this disclosure was made in the context of using database-searching and sequence comparison methods to find sequences that best match a given test sequence or to find the best alignment between sequences. After noting that sequence comparison is more of a qualitative science than a quantitative science (page 146), George et al. disclose that statistical measures of similarity do not necessarily reflect biological significance. Nonetheless, there is nothing in George et al. to support the assertion that the art worker would engage in undue experimentation to identify a peptide having a particular activity.

Thus, neither of the references put forth by the Examiner provide any evidence to support the proposition that the activity of an amino acid substituted peptide relative to the corresponding nonsubstituted peptide is unduly unpredictable.

It is respectfully submitted that the pending claims are in conformance with the

requirements of 35 U.S.C. § 112(1). Therefore, withdrawal of the rejection of the claims is respectfully requested.

The 35 U.S.C. § 102(b) Rejections

The Examiner rejected claims 1, 3-4 and 6-7 under 35 U.S.C. § 102(b) as anticipated by Rollins et al. (U.S. Patent No. 5,459,128). The Examiner also rejected claims 1 and 8-10 under § 102(b) as anticipated by Clark-Lewis et al. (Proc. Natl. Acad. Sci. USA, 90, 3574 (1993)). These rejections are respectfully traversed.

The mature form of MCP-1 has about 78 amino acids and attracts and activates monocytes. The Rollins et al. patent generally discloses MCP-1 "derivatives", i.e., MCP-1 derived polypeptides having amino acid substitutions or deletions, or insertions of an epitope tag, relative to the mature form of MCP-1. Preferred MCP-1 derivatives are disclosed as those with changes at amino acid position 3, 24 or 28, or which lack amino acids 2-8, of MCP-1. It is also disclosed that useful MCP-1 derivatives are those having decreased or no chemoattractant activity relative to MCP-1 and which inhibit the activity of MCP-1.

Figure 2 of the Rollins et al. patent shows the monocyte chemoattractant activity of 12 MCP-1 derivatives relative to wild-type MCP-1. Ten out of twelve of the derivatives had less than 60% of the chemoattractant activity of MCP-1. Four of the derivatives which had less than 10% of the chemoattractant activity of wild-type MCP-1 were tested in a dose response assay for their ability to inhibit monocyte chemotaxis in the presence of FX2, a derivative of wild-type MCP-1 having a carboxy-terminal epitope tag and 92% of the chemoattractant activity of wild-type MCP-1. The four derivatives were 7ND (a derivative which lacks residues 2-8 of MCP-1 and has a C-terminal epitope tag), D3A (a derivative with a D to A substitution at position 3 and a C-terminal epitope tag), R24F (a derivative with a R to F substitution at position 24 and a C-terminal epitope tag), and Y28D (a derivative with a Y to D substitution at position 28 and a C-terminal epitope tag). Only two of the derivatives, 7ND and Y28D, reduced MCP-1-induced migration in a dose dependent manner.

Although Rollins et al. indicate that in MCP-1 derivatives other than those which encode D at position 3, R at position 24, D at position 28, and a deletion of residues 2-8, of MCP-1 are envisioned, the only derivatives disclosed in Rollins et al. which lack or have decreased chemoattractant activity relative to MCP-1 and which inhibit the monocyte chemoattractant

activity of MCP-1 were Y28D and 7ND (see Figure 3 of Rollins et al.). Note that at higher concentrations, R24F and D3A increased MCP-1-induced migration of monocytes. Thus, the only derivatives in the Rollins et al. patent having the disclosed desired properties, i.e., no or reduced chemoattractant activity relative to MCP-1 and inhibition of MCP-1-induced migration, are derivatives that are about 71-78 amino acid residues in length and have modifications in the amino-terminal half of the mature form of MCP-1.

Rollins et al. do not disclose or suggest that a portion of a chemokine, e.g., a peptide of MCP-1, having less than 30 amino acid residues, would inhibit MCP-1-induced migration of monocytes. Therefore, the Rollins et al. patent does not anticipate Applicant's invention.

Clark-Lewis et al. relate the activity of modified forms of the chemokines PF-4 and IL-8. The amino acids at positions 4, 5 and 6 of the mature form of IL-8 (the mature form of human IL-8 has about 72 amino acid residues) are E, L and R, respectively (the "ELR" motif). The ELR motif had been shown to be required for IL-8 receptor binding and neutrophil activation. PF-4 (the mature form of which has about 70 residues) lacks the ELR motif. To determine whether the introduction of the ELR motif into PF-4 yields a molecule with altered specificity, various modified forms of PF-4 were tested for their neutrophil stimulatory and neutrophil chemoattractant activities. In contrast to full-length PF-4 and a modified form of PF-4 which lacked the amino-terminal 6 residues of PF-4 (DLQ-PF4), ELR-PF4 (in which residues 1-9 of PF-4 were replaced with ELR) stimulated neutrophils (as determined by elastase release) and induced neutrophil chemotaxis. ELR-PF4, but not [E^7,R^9]PF4 (a modified form of PF-4 in which positions 7 and 9 of PF-4 were substituted with E and R, respectively), competed with labeled IL-8 for binding to IL-8 receptors. Note that these modified forms of PF-4 were greater than 30 amino acids in length.

To determine whether the ELR domain had the characteristics of a free peptide, several linear ELR containing peptides (i.e., ELR, ELRA, AVCPRSAKELRA), and cyclic ELR containing peptides (i.e., CPGELRC, CPGGKELRC, CGPKELRC, CPGELRAQC, and CLPRSAKELRC), were tested in a competitive binding assay and a neutrophil chemotaxis assay. "[N]one competed for binding to the IL-8 receptor or had functional activity when tested at up to millimolar concentrations" (page 3576). The authors conclude that ELR is necessary but not sufficient for IL-8 receptor binding and neutrophil activation, and that IL-8 and other neutrophil stimulating proteins must interact with the receptor through additional binding sites to

ensure signaling.

Thus, Clark-Lewis et al. do not teach or suggest that linear or cyclic peptides of chemokines of 30 amino acids or less which lack the amino terminus (mature chemokines are generally about 70 amino acids in length and the ELR motif, if present, is generally within the first 10 amino acids of the mature form) would be biologically active. Therefore, Clark-Lewis et al. do not anticipate Applicant's invention.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney at the below-listed number to facilitate prosecution of this application. If necessary, please charge any additional fees deemed necessary to Deposit Account 19-0743.

Respectfully submitted,
DAVID J. GRAINGER ET AL.
By their Representatives,
SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6959

Date October 7, 1999 By _____
Janet E. Embretson
Reg. No. 39,665

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Assistant Commissioner of Patents, Washington, D.C. 20231 on **October 7th, 1999**.

GREG HANSON
Name

Signature